Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE RELATIVE RATE OF GROWTH OF MILK BACTERIA IN RAW AND PASTEURIZED CLEAN MILK.*

E. Q. St. John and M. E. Pennington

(From the Philadelphia Clinical Laboratory.)

The significance of the relative rate of growth of various organisms in raw and heated milk has, during comparatively recent years, brought out a considerable mass of work the results of which vary widely, as one would expect, considering the difficulties in obtaining material, in technique, and in overcoming or accounting for the many variations, small in themselves, but of importance when dealing with so delicate a problem in a medium so readily influenced. Hence, one is not surprised to find Fokker,[1] Hesse,[2] Schottelius,[3] Kitasato,[4] Friedrich,[5] Heinemann,[6] and others maintaining a germicidal activity for fresh cow's milk, which activity is destroyed by heat, and equally positive assertions on the part of Basenau,[7] Heim,[8] Kleimmer,[9] Conn and Esten,[10] and others that such a power is not manifested by raw milk; while the intermediate school is represented by Moro-Graz,[11] Schenk,[12] Cozzolino,[13] etc., who would assign such a property to milk from some animals and not to that from others.

Much of this variation of opinion we believe to be due to the conditions under which experiments were performed, notably, the method of obtaining the milk. Unless drawn from the cow in a sterile condition it cannot be rendered so without alteration, to a greater or less extent, of those substances upon which the "life" of the milk depends, and while possible it is difficult, first, to find a cow giving sterile milk and, then, to draw it in a sterile condition. However, such experiments have been made and the results obtained by sowing pure cultures of various organisms in sterile, fresh milk would indicate that such milk does inhibit growth for some organisms more and for some less strongly.[14]

While such experiments are of great value they leave untouched, except by inference, a crucial point in the practical pasteurization and sterilization of milk for infant-feeding or for the general public which the large city milk dealer is more and more frequently forcing

* Received for publication June 10, 1907

to the use of milk that has been subjected to the heating process misnamed "pasteurization." The point referred to is, "What is the effect of heated milk on a mixture of various varieties and numbers of those organisms commonly inhabiting milk and whose source is not only the cow but air, water, soil, dust, etc.

After commercial heating milk is almost invariably reinfected with a variety of organisms before it leaves the hands of the dealer,[16] and is subject to many sources of infection in the ordinary household before it is consumed. The work of isolating in pure culture the organisms inhabiting a number of specimens of milk is appreciated only by those who have tried it; and the returning of such cultures to the heated milk in their original quantity, quality, and relative proportions is a feat which has not been, and is not likely to be, readily accomplished. The addition of a portion of raw milk, with its accompanying organisms, to a portion of the same milk previously heated is open to objection because it either greatly reduces the actual number of organisms per c.c. which, according to Hunziker,[15] makes a difference in germicidal activity, or it carries in a goodly quantity of raw, unchanged milk which may modify the final result.

Because of the importance of the practical side of this question, that is, whether the commonly occurring mixtures of milk organisms show a different rate of growth in raw and in heated milk, we decided to devise a scheme by which the organisms, in like number and species and unchanged in activity by isolation on artificial media, could be studied side by side in the fresh, clean, raw product, and in the fresh, clean, pasteurized milk.

This we accomplished by centrifuging raw milk at a high rate of speed until the majority of the organisms had settled at the bottom of the tube. Plating the supernatant milk showed that from 50 to 75 per cent of them were thrown down after 15 minutes, whirling at a speed somewhat above 3,000 revolutions per minute. By carefully pipetting off the milk the sediment could all be kept in a volume less than 1 c.c. It was then transferred to milk which had been heated under the conditions desired. A sufficient amount of residue was added to supply the heated milk with approximately its original quota of organisms. Of course, due precautions were observed throughout to prevent contamination from handling.

That the reinfection gives a milk bacteriologically comparable with the raw product is proven not only by the total initial count, but by the proportions of acid-formers and liquefiers present in both samples, which, as the tables show, are practically identical.

The heating of the milk was carried out in small flasks at a temperature of 79° C. for 20 minutes, cooling quickly in running water and finally on ice. It will be noticed that the germs surviving were very few. As soon as it was cold it was reinfected and both samples were then plated.

The counts were made on plain agar, litmus-lactose agar, and litmus-lactose gelatin. The gelatin plates, showing the liquefying species as well as the acid-formers and general count, were, of course, kept at 20° C. The agar plates were grown for 24 hours at 37° C., then for two days at room temperatures. We find this initial high temperature gives clear-cut colonies, an especially desirable condition when acid-formers are to be counted.

The three samples to be compared, (*a*) raw, (*b*) heated and reinfected, (*c*) heated, were then kept either at room or refrigerator temperatures and plated at intervals of 24 hours until curding took place.

This happened, ordinarily, in from 72 to 96 hours for the pasteurized-reinfected milk, at which time the raw milk was still sweet and remained so for at least 24 hours longer, making the life of the raw milk, as determined by curding, considerably longer than that of the pateurized-reinfected milk. While the life of all the samples at room temperature was shorter than when kept in the ice-box, the relative differences were still well marked.

Counts made of these samples as above outlined showed a very marked increase in the bacterial content of the pasteurized-reinfected samples as compared with the raw. This increase ordinarily was plainly visible at the end of the first 24 hours, though if kept very cold there was an occasional decrease for that length of time, which decrease was more plainly marked in the raw than in the heated sample. In one case only, out of all the experiments made, was this decrease maintained for a longer period (Exp. 5, Table 1), and the whole course of this milk in all three samples differs so widely from the rest that too much stress cannot be laid upon it. It is given.

principally, to show what wide variations may occur in individual cases.

The decrease during the first day in these experiments is of additional interest because the milk was between six and eight hours old when we received it and its ability to kill organisms is, therefore, of fairly long duration. Prior to its delivery at the laboratory, however, its history was one of most unusual cleanliness and care. It was drawn from cattle inspected monthly by a veterinarian, tuberculin tested twice yearly; each individual cow was examined weekly for evidences of udder inflammation, as shown by the presence of pus or streptococci or staphylococci in quantity in her milk. The most rigorous practices regarding cleanliness of barns and cattle are followed, including a separate room for milking. The cooling of the milk is to below 40° F., generally 34° to 36°, and is accomplished so promptly that scarcely five minutes elapse between the drawing of the milk and its final sealing in sterile bottles, with sterile caps made flush with the bottle top with paraffin to prevent contamination while in transit to the consumer. The milk was packed in ice for transportation. If a germicidal property is an attribute of *clean* milk, as Hunziker[15] states, it should certainly find expression in milk so produced.

If, on the other hand, as Stocking[10a] asserts, "The decrease in the numbers of the normal milk bacteria during the first few hours after milking is not properly to be attributed to a germicidal condition or property possessed by the milk, but simply to the natural dropping-out of those species which do not find the milk a suitable medium in which to develop," the returning to the heated milk of the unchanged organisms should still be followed by the same decrease, provided the heating has in no wise changed its behavior as a culture medium.

Aside from this decrease in the first few hours, however, there remains the question of the relative rate of increase of the organisms in the raw and heated milk, a question which, as we said before, is of vital interest when considered from the standpoint of infant-feeding.

Table 1 gives five experiments made as above outlined, the results being expressed as ratios of increase, taking the initial count as unity. Accordingly, we find, in Exp. 1 that after 48 hours at room temperature the raw milk has increased its original content 1,355 times; the pasteurized milk has 51,904 times as many as in the beginning. In Exp. 3, after 96 hours in the ice-box, the raw milk has multiplied 1,328 times; while the pasteurized and reinfected has 34,550 times as many as in the beginning.

TABLE 1.

RATIO OF INCREASE OF GERM CONTENT.

	Number of Hours	Raw Milk	Pasteurized and Reinfected Milk
Experiment 1— Room temperature	Immediately	1:1	1:1
	24 hours	1:29	1:159
	48 "	1:1355	1:51,904
Experiment 2— Room temperature	Immediately	1:1	1:1
	24 hours	1:7	1:67
	48 "	1:55	1:35,000
Experiment 3— Ice-box temperature	Immediately	1:	1:
	24 hours	1:4	1:15
	48 "	1:218	1:637
	72 "	1:967	1:2700
	96 "	1:1328	1:34,550
Experiment 4— Ice-box temperature	Immediately	1:	1:
	24 hours	Loss of organisms	Decrease in count
	48 "	1:1.1	1:2
	72 "	1:3	1:12
	96 "	1:8	1:239
Experiment 5— Ice-box temperature	Immediately	1	1:
	24 hours	Decreasing count for 24 hrs.	Decreasing count for 48 hrs.
	48 "	1:1.8	
	72 "	1:1.3	1:1.6
	96 "	1:2.5	1:6
	120 "	1:22	1:84
	144 "	1:222	1:1703
	168 "	Sour	1:2222

Experiment 6 gives in greater detail the work leading to such figures as are summarized in Table 1. In this case the acid colonies were differentiated by counting on litmus-lactose agar and their record appears in the specified column. The milk was kept at 15° C. during the experiment.

Experiment 7 shows not only the course of the acid-formers, but also those organisms liquefying gelatin, a group of great importance in heated milk. The temperature here was 15° C.

Both of these experiments follow the usual course—a marked increase in the number of organisms in the heated milk. This is not, apparently, confined sharply to either the acid-forming or peptonizing groups, sometimes one, sometimes the other being in excess. However, at the close of the experiments, the peptonizing organisms are generally ahead in the heated milk.

EXPERIMENT 6.

	Raw Clean Milk				Pasteurized Clean Milk Reinfected				Pasteurized Milk
Number of Hours	Total Number of Organisms per c.c.	Ratio of Increase	Total Number of Acid-Formers per c.c.	Ratio of Increase	Total Number of Organisms per c.c.	Ratio of Increase	Total Number of Acid-Formers per c.c.	Ratio of Increase	Total Number of Organisms per c.c.
Immediate.....	7,500	1:1	3,500 46%	1:1	7,200	1:1	2,600 36%	1:1	10
24 hours.......	51,000	1:6.6	13,000 25%	1:3.6	130,000	1:18	55,000 42%	1:21	..
48 "	2,000,000	1:266	500,000 25%	1:142	3,600,000	1:500	500,000 14%	1:192	..
72 "	288,000,000	1:38,400	84,000,000 29%	1:24,000	460,000,000	1:63,888	94,000,000 20%	1:36,153	..

EXPERIMENT 7.

	Raw Clean Milk						Pasteurized Clean Milk Reinfected					
Number of Hours	Total Number of Organisms per c.c.	Ratio of Increase	Total Number of Acid-Formers per c.c.	Ratio of Increase	Total Number of Liquefiers per c.c.	Ratio of Increase	Total Number of Organisms per c.c.	Ratio of Increase	Total Number of Acid-Formers per c.c.	Ratio of Increase	Total Number of Liquefiers per c.c.	Ratio of Increase
Immediate...	1,370	1:1	650 47.5%	1:1	150 10.9%	1:1	1,250	1:1	540 43.2%	1:1	150 12%	1:1
24 hours.....	2,300	1:1.6	650 28.2%	1:1	200 8.65%	1:1.3	2,200	1:1.7	200 9.0%		600 27%	1:4
48 "	210,000	1:91	82,000 39%	1:126	64,000 30%	1:426	514,000	1:411	350,000 68%	1:648	200,000 46%	1:2,933
72 "	5,900,000	1:4,304	104,000 1.7%	1:601			11,700,000	1:9,360	660,000 5.5%	1:1,222		

EXPERIMENT 8.

	RAW CLEAN MILK 80 HOURS OLD						PASTEURIZED CLEAN MILK REINFECTED 80 HOURS OLD: SEDIMENT WASHED						PASTEURIZED
Number of Hours	Total Number of Organisms per c.c.	Ratio of Increase	Total Number of Acid-Formers	Ratio of Increase	Total Number Liquefiers per c.c.	Ratio of Increase	Total Number of Organisms per c.c.	Ratio of Increase	Total Number of Acid-Formers per c.c.	Ratio of Increase	Total Number of Liquefiers per c.c.	Ratio of Increase	Number of Organisms per c.c.
Immediate..	80,000	1:1	13,000 16%	1:1	2.400 3%	1:1	10,000	1:1	4.100 41%	1:1	300 3%	1:1	20
24 hours....	7,600,000	1:95	540.000 7%	1:41	100,000 1.3%	1:41	7,050,000	1:705	2,100.000 29%	1:512	110.000 1.5%	1:366	..
48 "	25,800,000	1:322	8,600.000 33%	1:661	6,000.000 23%	1:2,500	28,000,000	1:2,800	13,000,000 46%	1:3,170	3,000,000 10%	1:13,333	..
72 "	264,000,000	1:3.300	80,000,000 30%	1:6,153	8,000,000 3%	1:3,333	257,000,000	1:25,700	55,000,000 21%	1:13,414	7,000,000 2.7%	1:23,000	..
96 "	42,000,000	1:525	11,000.000 26%	1:846	20,000.000 47%	1:8,333	67,000,000	1:6,700	12,000,000 17%	1:2,926	35,000,000 52%	1:116,666	..

MILK FROM MILK SHOP—NO HISTORY

	Raw Milk						Pasteurized Milk Reinfected						Pasteurized Milk
	Total Number of Organisms per c.c.	Ratio of Increase	Total Number of Acid-Formers per c.c.	Ratio of Increase	Total Number of Liquefiers per c.c.	Ratio of Increase	Total Number of Organisms per c.c.	Ratio of Increase	Total Number of Acid-Formers per c.c.	Ratio of Increase	Total Number of Liquefiers per c.c.	Ratio of Increase	Total Number of Organisms per c.c.
Exp. 9 Immediate..	23,000	1:1	8,400 36%	1:1	5,000 21.3%	1:1	17,000	1:1	5,000 35%	1:1	4,000 23%	1:1	40
24 hours....	1,620,000	1:70	......	....	600.000 37%	1:120	2,320,000	1:136	......		800,000 38%	1:200	..
48 "	10,000,000	1:434	4,600,000 46%	1:547	5.000,000 50%	1:1,000	3,200,000	1:247	1,200,000 37.5%	1:240	1,800,000 56%	1:450	..
Exp. 10 Immediate..	500,000	1:1	200,000 40%	1:1	400,000 80%	1:1	300,000	1:1	120,000 40%	1:1	40,000 13%	1:1	..
24 hours....	107,000,000	1:214	82,000,000 76%	1:410	45,000,000 42%	1:112	100,000,000	1:333	50,000,000 50%	1:416	30,000,000 30%		
48 "	Sour						Sour						

Centrifuging and returning the sediment to pasteurized milk does, of course, enrich the milk in serum to some extent. Lest such an addition should affect in any way the growth of the bacteria we washed the sediment in sterile physiological salt solution several times before sowing the milk to be replenished—thereby removing this factor, if it be one. The experiment is outlined in No. 8. This milk also differed from the others in that it was 80 hours old when used, having been kept on ice to see whether the age factor, after the first few hours, made any great difference in the results obtained.

Apparently the amount of serum which this method adds to the milk does not alter the relative rate of growth of the organisms; for, though the initial count in the reinfected milk is lower than in the raw, due, of course, to the difficulty of washing and preserving the number of organisms intact, the ratio of increase in the heated milk is far in excess of the unheated. Originally, however, this milk was very clean and the temperature was low throughout.

What the effect of heat would be on an ordinary shop milk was the next question raised. But two experiments have been made, and they are not sufficient to give an adequate reply to the above query. The results are appended and we plan further work along this line with ordinary milk for which a history can be obtained.

CONCLUSIONS.

The foregoing experiments would indicate that aside from the lowering of the numbers of organisms in milk during the first few hours, be that due to a germicidal activity, or to unnatural environment, there is a restraining power for ordinary milk organisms in raw milk lasting at least to the curding point. This condition is either not present or is reduced in milk which is heated to 79° C., at a temperature below that required to destroy its oxydizing action.

This restraining power seems to apply to the organisms commonly found in milk in the combinations ordinarily present there. A complete qualitative study of the flora inhabiting the samples of milk cited was not carried through, but the predominating and most commonly occurring forms were isolated and proven to be *Bact. aerogenes*, *B. solitarius* (Ravenel), *B. formosus* (Ravenel), *M.*

aurantiacus, Bact. fulvum (Zimmermann), *M. viticulosis,* and *M. lactis.*

It is an accepted fact by many doing laboratory work on milk that pasteurization or sterilization of milk and its subsequent reinfection may be attended by serious consequences in the child, and, therefore, it is urged that even greater care in handling and refrigeration be employed for heated milk than for the raw article.

Such precautions, so far as a clean milk is concerned, would seem to be strongly emphasized by the foregoing experiments. They afford additional light, also, on the milk "pasteurized" commercially, which is reinfected after heating and generally, in cities, kept the better part of 24 hours before delivery to the consumer, who uses it at intervals during another 24 hours and who may heat it a second time.

REFERENCES.

1. FOKKER. *Zeit. f. Hyg.*, 1890, 9, p. 41.
2. HESSE. *Zeit. f. Hyg.*, 1894, 17, p. 238.
3. SCHOTTELIUS. *Centralbl. f. Bact.*, 1896, 20, p, 897.
4. KITASATO. *Zeit. f. Hyg.*, 18, 5, p. 492.
5. FRIEDRICH. *Arbeiten a. d. kaiserl. Gesundheit.*, 1896, 13, p. 465.
6. HEINEMANN. Abstract read at meeting of A.A.A.S., Dec., 1906.
7. BASENAU. *Arch. f. Hyg.*, 1895, 21, p. 44.
8. HEIM. *Arbeiten a. d. kaiserl. Gesundheit.*, 1889, 5, p. 294.
9. KLIMMER. *Arch. f. Kinderheilk.*, 1903, 36, p. 1.
10. CONN AND ESTEN. *Storr's Agri. Exp. Sta. Rep.*, 1904, 16, p. 27.

10*a*. STOCKING. *Storrs Agric. Exp. Sta. Rep.*, 1904, 16, p. 89.

11. MORO-GRAZ. *Arch. f. Kinderheilk.*, 1900, 33, p. 435.
12. SCHENK. *Monatsschr. f. Geb. u. Gyn.*, 1904, 19, Nos. 2–4.
13. COZZOLINO. *Arch. f. Kinderheilk.*, 1900, 33, p. 405.
14. EVANS. *Trans. Philadelphia Pathological Society*, 1905.
15. HUNZIKER. *Cornell University Agric. Exp. Sta. Bull.*, 1901.
16. PENNINGTON AND MCCLINTOCK. *Am. Jour. Med. Sci.*, 1905, 130, p. 140.